Claims

1. Method for generating transgenic eukaryotic cells having a modified Rosa26 locus, which method comprises (a) introducing a functional DNA sequence into the Rosa26 locus of starting eukaryotic cells, wherein said functional DNA sequence is a gene expression cassette comprising a gene of interest operatively linked to a promoter or is a DNA sequence which can be converted into such gene expression cassette.

2. The method of claim 1 wherein the functional DNA sequence is introduced into the eukaryotic cells

by homologous recombination with a targeting vector comprising said functional DNA sequence flanked by DNA sequences homologous to the Rosa26 locus, or

by site specific recombinase mediated recombination with a recombination vector comprising said functional DNA sequence flanked by a pair of first recombinase recognition sites (RRSs).

3. The method of claim 1 or 2, wherein the eukaryotic cells

(i) are derived from a multi-cell organism including vertebrates, invertebrates and plants, preferably are vertebrate cells, more preferably are derived from a mammal, including rodents such as mouse, rat, etc., or a fish such as zebrafish; and/or

(ii) are primary cells or immortalized cells;

most preferably the cells are mammalian embryonic stem (ES) cells.

4. The method according to any one of claims 1 to 3, wherein

(i) the gene of interest is selected from recombinases, reporter genes, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, disease causing gene products, toxins, etc.; and/or

(ii) the promoter is a heterologous promoter and preferably is a ubiquitous or tissue specific promoter, either constitutive or inducible, preferably is a CAGGS, hCMV, PGK, FABP, Lck, CamKII, CD19, Keratin, Albumin, aP2, Insulin, MCK, MyHC, WAP, Col2A, Mx, tet or Trex promoter; and/or

(iii) the functional DNA sequence or gene expression cassette further comprises one ore more additional functional sequences including but not limited to marker genes, second recombinase recognition sites differing from the first recombinase recognition sites, poly A signal, introns, etc.; and/or

(iv) the targeting vector and recombination vector further comprises tags for protein detection, enhancers, selection markers, etc.

5. The method according to anyone of claims 2 to 4 which comprises homologous recombination, wherein the DNA sequences homologous to the Rosa26 locus are 0.2 to 20 kB, preferably 1 to 10 kB long.

6. The method of claim 5, wherein the transgenic eukaryotic cells are derived from mouse, and wherein

(i) the DNA sequences homologous to the Rosa26 locus are derived from the 5' and 3' flanking arm of the mouse Rosa26 locus, preferably said homologous DNA sequences having the sequences shown in SEQ ID NO:4 and 5, respectively, and/or

(ii) the promoter is a CAGGS-promoter,

most preferably the targeting vector has the sequence shown in SEQ ID NO:7.

7. The method according to anyone of claims 2 to 4 which comprises recombinase mediated recombination and which comprises the steps of

(a1) introducing into the starting cells an acceptor DNA which integrates into the genome of the starting cell, the acceptor DNA comprising two mutally incompatible first RRSs, and introducing into the therewith obtained cell

(a2) a donor DNA comprising the same two mutually incompatible first RRSs contained in the acceptor DNA by utilizing a recombination vector as defined in claims 2 to 4; and

(a3) the recombinase which catalyzes recombination between the RRSs of the acceptor and donor.

8. The method of claim 6, wherein

(i) the RRS are loxP or FRT sites or variants thereof; and/or

(ii) the acceptor DNA comprises a negatively selectable marker gene;

(iii) the donor DNA comprises an inactive positive selection marker.

9. The method according to any one of claims 1 to 8, preferably according to claims 2 to 6, which further comprises one or more of the steps

(b) isolating the eukaryotic cells, preferably the ES cells having the desired functional DNA sequence integrated into the Rosa26 locus; and/or

(c) modifying the integrated functional DNA sequence and isolating ES cells having the desired modified functional DNA sequence.

10. A targeting vector as defined in claims 1 to 8, preferably as defined in claims 2 to 6.

11. A eukaryotic cell having a modified Rosa26 locus obtainable by the method of claims 1 to 9.

12. A method for preparing transgenenic multi-cell organism having a modified Rosa26 locus which comprises utilizing the method as defined in claims 1 to 9.

13. The method of claim 12, wherein the transgenenic multi-cell organism is a non-human mammal and said method comprises modifying an ES cell as defined in claims 1 to 9.

14. The method of claim 12 or 13 which further comprises one or more of the steps

(d) injecting ES cells obtained in steps (b) or (c) into blastocysts; and/or

(e) generating transgenic non-human animals carrying one or more functional genes of interest at the Rosa26 locus.

15. A transgenic multi-cell organism and a transgenic non-human mammal obtainable by the method of claims 12 to 14, respectively, and having an operatively functional gene expression cassette integrated into its Rosa26 locus.

16. Use of the eukaryotic cell of claim 11, the transgenic multi-cell organism of claim 15, or the transgenic non-human mammal of claim 15 for gene function studies, drug development, as disease model animals, etc.